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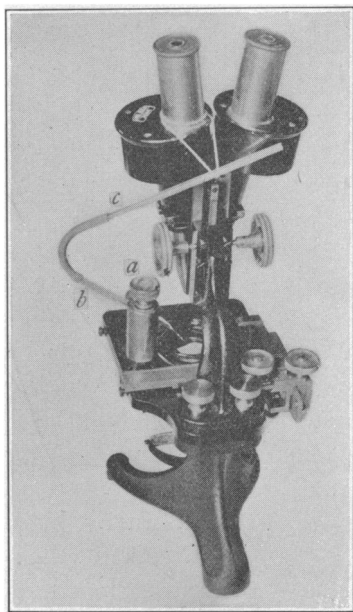
# EXPERIMENTS ON THE EGGS OF CHÆTOPTERUS AND ASTERIAS IN WHICH THE CHROMATIN WAS REMOVED.

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Last July and August, while in the U. S. Bureau of Fisheries Laboratory at Wood's Hole, Mass., I devised an apparatus for removing blastomeres from ascidian eggs, but as the chorion of these eggs was too tough for a successful operation, I tried to use the apparatus for other purposes. I found that with it I could remove parts of the unsegmented eggs of echinoderms and annelids, and, in the few weeks at my disposal, performed the experiments mentioned below.

## THE APPARATUS.

To a Greenough binocular stand (see accompanying figure) I attached a Spencer mechanical stage supplied with a fine adjustment screw (*a*) made by Wieback and Pietzsch, Philadelphia. The addition of this screw allowed movement in three dimensions, and the fine adjustment (*a*) carried a tube (*b*) drawn to a capillary opening at the lower end, and with the upper end attached by a rubber tube to the glass tube (*c*), which I held in my mouth during the operation. The lenses used were Zeiss binocular objective  $\alpha_3$  and a pair of Zeiss orthomorphic oculars, 4, giving a magnification of 65 diameters. Eggs were placed in 2–3 mm. depth of sea water in a glass dish on the stage of the microscope and the capillary end of the tube (*b*) inserted into the water above



them and allowed to remain until the water rose in it as high as it would by capillarity. The dish was moved until an egg of the most favorable orientation was brought in the center of the field. The capillary tube was then brought against the proper spot on the egg and a portion of the egg sucked away through the tube (*c*) which was held in the mouth. Immediately the operated egg was lifted from the dish by means of a capillary pipette just large enough to admit it easily, and placed in a watch glass half full of sea water. This capillary pipette does not need a bulb, as water enters it by capillarity. The size of the capillary end of the tube (*b*) was determined for the egg of each species by trial. Some parts of the mechanical stage were an unnecessary hindrance and were removed.

#### EXPERIMENTS ON THE EGGS OF CHÆTOPTERUS PERGAMENTACEUS CUVIER.

Mead ('98<sup>2</sup>) and J. Loeb ('01) found that differentiation went on in the unfertilized egg of *Chaetopterus* in solutions of KCl, and F. R. Lillie showed that this differentiation bears a semblance to the normal development save that cell division is usually suppressed.

I removed the chromosomes during the maturation divisions of the unfertilized egg in KCl solutions by sucking away the part of the egg immediately under the first polar body, in order to determine the effect of the chromatin on differentiation. A small percentage of eggs were so injured by the operation that they remained inactive until disintegration. Others, however, performed those "amoeboid" movements which Loeb mistook for cleavage, just as in operated eggs. Further than this no changes were visible externally, but sections showed that irregular flowing of substances had taken place within the egg. None of the operated eggs developed cilia, and we might conclude that the chromatin is necessary for the formation of cilia, a function that has been attributed to the archoplasm. But as the centrosomes were probably removed with the chromosomes, and as the element of injury could not be excluded, this negative evidence should not be considered conclusive. Nearly one hundred eggs were operated on, and, although I do not think it advisable to

record the individual experiments, I will state the precautions against error that were used :

The majority of the *Chætopterus* females were gathered by me, or under my direct supervision, and were never placed in the same vessel with males. Each female was washed in a stream of fresh water before eggs were removed from it. Except during two days no males were allowed in the same laboratory with the females. One check of eggs in sea-water and another in sea-water to which KCl had been added, were kept to each experiment. None in the check in plain sea-water developed, while many in the KCl sea-water developed cilia. In each lot of eggs the operations were continued from the first appearance of the first polar body to the formation of the second polar body, and sometimes a few minutes later. Some eggs were fixed and sectioned immediately after the operation, others at later intervals up to twenty-four hours, and in this way was determined whether any chromatin had been left in the egg.

#### EXPERIMENTS ON THE EGGS OF *ASTERIAS FORBESII*.

Eggs from which the chromosomes were removed and eggs from which the whole nucleus was removed were fertilized with sperm of the same species. These operated eggs did not show any differences from the normal save in number of chromosomes, in increased tendency toward polyspermy and in increased mortality. This last prevented my ascertaining whether any differences would appear in the later development.

An attempt was made to remove some of the chromosomes during the first cleavage, but the spindle was so much more viscid than the surrounding yolk that no part of it could be sucked out without removing all of it. In some sections the spindle was shown pulled to the surface of the egg but not very much distorted.

Eggs from which chromosomes were removed were mixed with sperm of a species of *Synapta*, but did not show signs of development save for the separation of the "fertilization membrane" from the egg.

In the experiments on the *Asterias* egg the following precautions were used: The starfish were washed in a strong stream of

fresh water before removal of the sexual organs, and after a male had been opened my instruments and hands were washed in like manner, to remove all spermatozoa. A check of unfertilized eggs was always kept, and in case some eggs developed in the check, the material was thrown out. In a number of experiments the eggs were fixed and sectioned as a further check.

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